

Study of the Effect of Hydro-Ethanollic Extract of *Commiphora africana* (Stem-bark) on Inflammation and Pain in Rodents

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Abstract: Inflammatory response of living bodies is a protective mechanism against infected organisms. The aim of this study was to evaluate the effect of hydro-ethanollic extract of *Commiphora africana* (stem-bark) on inflammation and pain. The anti-inflammatory activity was investigated using the rat paw edema model. The analgesic activity was studied using the acetic acid induced writhings in mice. The results showed that the hydro-ethanollic (stem-bark) extract of *Commiphora africana* possess significant ($p < 0.05$) anti-inflammatory effect and inhibit abdominal constriction caused by acetic acid in mice. The results of the preliminary phytochemical screening of *Commiphora africana* (stem bark) extract revealed the presence of flavonoids, tannin, anthraquinone, cardiac glycosides, triterpenoids, saponins, alkaloids and reducing sugars. The LD₅₀ was calculated as 3708.7 mg/kg in rats and 471.2 mg/kg in mice. It was concluded that the plant is or may be a very good source of analgesic.

Key words: Analgesic, anti-inflammatory, *Commiphora africana*, phytochemical screening

INTRODUCTION

Many medicinal plants are used in developing countries for the management of pain and inflammatory conditions. The validation of the folkloric claims of these medicinal plants will provide scientific basis for the conservation of tropical medicinal resources, the deployment of the beneficial ones as phytomedicine in the primary healthcare and the development of potential bioactive constituents

Commiphora africana belongs to the family of Burseraceae and a group of plant called Myrrh (Hanus *et al.*, 2005). It is a small tree, but usually not more than 5 m high. It can be recognized unmistakably from a distance by its outline- a spherical top and a short trunk with low branches. The anti-lipidaemic, anti-cholesterolaemic and anti-atherosclerotic properties of a number of *Commiphora* species have been extensively studied (Michie and Cooper, 1991; Newall *et al.*, 1996). The ethanolic leaf extract of *Commiphora africana* has also been demonstrated to possess an anti-lipidaemic property (Adebayo *et al.*, 2006). *Commiphora myrrh* has been shown to exhibit hypolipidaemic activity (Malhotra *et al.*, 1977). The antilipidaemic activity of *Commiphora mukul* (guggulipid) has been established (Wang *et al.*, 2004). It exerts effective lipid-lowering activity by reducing total cholesterol, Very Low Density Lipoproteins (VLDL), and Low Density Lipoproteins (LDL) cholesterol, while elevating High Density

Lipoproteins (HDL) cholesterol level (Wang *et al.*, 2004). The effects of *Commiphora* leaf extract on some biomedical markers of liver and kidney have also been reported (Aliyu *et al.*, 2002). There is a dearth of information on anti-inflammatory and anti-nociceptive, of *Commiphora africana* in the available literature hence the need to investigate the above-mentioned parameters.

MATERIALS AND METHODS

Animals: Swiss albino mice (weighing between 19 to 30g) and Wistar rats (weighing between 100 to 150g) of both sexes were used. They were obtained from the Animal House of the Department of Pharmacology and Clinical Pharmacy, ABU, Zaria. The animals, maintained on Excel feeds, Ilorin and water *ad libidun*, were kept in plastic cages at room temperature throughout the study. The experiment was conducted in a quiet laboratory between the hours of 900 and 1600 h.

Plant material: Samples of the stem bark of *Commiphora africana* was collected in the month of February 2009 within main campus of the Ahmadu Bello University (ABU) Zaria. The plant was identified and authenticated by M. Musa of the herbarium section of the Department of Biological Science, Ahmadu Bello University, Zaria, where a herbarium specimen (N_{o.} 900300) was deposited for future reference.

Preparation of the plant extract: The stem bark of *Commiphora africana* was collected and dried under shade and ground into powder. The powder (500 g) was macerated in 30 % of distilled water and 70% ethanol at room temperature for 24 h. It was then filtered using a filtered paper (Whatmann size no.1), and the filtrate evaporated to dryness in water bath at 60°C. A brownish residue weighing 30.5 g was obtained. This was kept in airtight bottle in a refrigerator until used.

Acute toxicity test: The lethal dose (LD₅₀) of the plant extract was calculated by the method of Lorke (1983) using 12 rats. In the initial phase, male and female Wistar rats were divided into three groups of three rats each. They were treated with the *Commiphora africana* stem bark extract at doses of 10, 100 and 1000 mg/kg per intraperitoneally (i.p.). Animals were observed for 24 h for signs of toxicity. No mortality was recorded. In the second phase of the toxicity study, the animals were divided into three groups of one rat each. They were treated with the *Commiphora africana* stem bark extract at doses of 1600, 2900 and 5000 mg/kg (i.p.). The median lethal dose (LD₅₀) was calculated using the second phase.

Phytochemical Screening: The preliminary Phytochemical screening of *Commiphora africana* extract (stem bark) was carried out in order to ascertain the presence or absence of various constituents utilizing standard conventional protocol (Trease and Evans, 1983; Harbone and Baxter, 1993).

Drugs used: All chemicals and drugs were obtained commercially and were of analytical grade.

(a) Anti-inflammatory studies: 25 Adult Wistar rats were divided into 5 groups of 5 rats in each group. The first group served as negative control (normal saline i.p.) the second, third and the fourth groups received different doses of the extract (50,100 and 200 mg/kg body weight i.p.) respectively while the fifth group-received indomethacin (20 mg/kg i.p.). After 30 min all the groups were administered 50 µg of a 2.5% solution of formalin, subcutaneously under the plantar surface of the left hind-paw. Any increase in the rats hind-paw linear diameter induces by the sub-plantar injection of formalin was taken as a measure of acute inflammation (Winter *et al.*, 1963). The difference between the readings at time 1 hour and different time intervals was taken as the thickness of edema.

$$\text{Inhibition (\%)} = \frac{\text{Mean paw diameter (control)} - \text{mean paw diameter (treated)}}{\text{Mean paw diameter (control)}} \times 100$$

(b) Acetic acid-induced writhings in mice: The test was conducted using the method described by Koster *et al.*, (1959). 25 Swiss albino mice were divided

into 5 groups of 5 mice each. Group 1 served as negative control (distilled water: 1.0 ml/kg i.p.) while groups two, three and four received the extract at doses 50, 100 and 200 mg/kg body weight i.p., and group five (positive control) received piroxicam at a dose of 20 mg/kg i.p., After 30 min all the groups were treated with acetic acid (0.06% 1.0 ml/100g i.p.). Mice were placed in individual cages. The number of abdominal contractions was counted 5 min after acetic acid injection for a period of 10 min; percentage inhibition of writhing was obtained using the formula

$$\text{Inhibition (\%)} = \frac{\text{mean number of writhing (control)} - \text{mean of writhing (test)}}{\text{Mean number of writhing (control)}} \times 100$$

Statistical analysis: All data were expressed as mean ± SEM. The data were analyzed statistically using one-way analysis of variance (ANOVA) with multiple comparisons versus control group. Values of p<0.05 were taken as significant (Duncan *et al.*, 1977).

RESULTS

Phytochemical analysis: Freshly prepared extract was subjected to preliminary Phytochemical screening test for various constituents. This revealed the presence of flavonoids, tannin, anthraquinone, cardiac glycosides, triterpenoids, saponins, alkaloids and reducing sugars.

Acute toxicity studies (LD₅₀): The sign of toxicity were first noticed after 8-10 h of extract administration. There was decrease locomotor activity, decrease feed intake, and prostration after 8 h of extract administration. The median lethal dose (LD₅₀) in rats was calculated to be 3708.7 mg/kg and in mice 471.2 mg/kg body weight intraperitoneally.

Table 1 The effect of hydroethanolic extract of *Commiphora africana* on formalin-induced hind paws edema in Wistar rats. The administration of 50 mg/kg of the extract lowered the paw volume in the experimental group when compared to the normal saline group. There was a statistically significant difference in the mean values of both variables at all time, p<0.05. A decrease in paw diameter was also observed when the extract was administered at the dose of 100 mg/kg compared to the normal saline group.. Similarly, the extract at 200 mg/kg showed significantly (p<0.05) higher values at all times when compared to the normal saline group.

Table 2 percentage inhibition expressed by the extract and indomethacin on formalin-induced edema in rats. 200 mg/kg of the extract showed a higher % inhibition of about 38.2% while the indomethacin group showed % inhibition of about 50.4.

Table 3 Effect of hydroethanolic extract of *Commiphora africana* on acetic acid-induced writhings in mice. The extract (200 mg/kg i.p.) exhibited a significant

Table 1: Effect of hydro-ethanolic stem-bark extract of *Commiphora africana* on formalin-induced edema in rats (n =25)

Treatments	Paw diameter (mm) at various times (min)					
	0 h	1 h	2 h	3 h	4 h	6 h
Normal saline 1 ml/kg	0.73±0.03 _{ns}	0.81±0.02 _{ns}	0.79±0.03 _{ns}	0.79±0.03 _{ns}	0.82±0.03 _{ns}	0.78±0.03 _{ns}
50 mg/kg	0.60±0.02 ^a	0.64±0.01	0.64±0.01 ^a	0.65±0.01 ^a	0.66±0.00 ^b	0.67±0.03 ^a
100 mg/kg	0.57±0.03 ^c	0.58±0.02 ^c	0.57±0.03 ^c	0.57±0.03 ^c	0.55±0.03 ^c	0.55±0.02 ^c
200 mg/kg	0.48±0.03 ^c	0.49±0.02 ^c	0.49±0.02 ^c	0.49±0.01 ^c	0.49±0.03 ^c	0.47±0.02 ^c
Piroxicam 20 mg/kg	0.38±0.01 ^c	0.40±0.00 ^c	0.40±0.00 ^c	0.41±0.01 ^c	0.38±0.01 ^c	0.37±0.01 ^c

Values are given as mean ± SEM; experimental groups were compared with control. Values are statistically significant at a = p < 0.05, b = p < 0.01, c = p < 0.001, ns= not significant

Table 2: Percentage inhibition expressed by the extract and indomethacin on formalin-induced edema in rats (n = 25)

Treatment	Dose (mg/kg)	Inhibition (%) at various times (h)						Mean % inhibition
		0 h	1 h	2 h	3 h	4 h	6 h	
Extract	50	17.8	12.3	12.3	10.9	9.6	8.2	11.8
Extract	100	21.9	28.3	27.8	27.8	32.9	29.5	28.0
Extract	200	34.2	39.5	37.9	37.9	40.2	39.7	38.2
Indomethacin	20	47.9	50.6	49.4	48.1	53.7	52.6	50.4

Table3: Effect of hydroethanolic extract of *Commiphora africana* on acetic acid-induced writhings in mice.

Treatment	Dose (mg/kg)	Mean of writhings	Inhibition (%)
Normal saline	1 ml/kg	17.4±6.16	
Extract	50	6.80±0.58 _{ns}	60.9
Extract	100	4.00±1.34 _{ns}	77.0
Extract	200	2.00±0.55 ^a	88.5
Piroxicaam	20	2.20±0.73	87.4

Values are given as mean ± SEM; experimental groups were compared with control. Values are statistically significant at a = p<0.05, ns = not significant

(p<0.05) inhibition of nociceptive induced by acetic acid in mice when compared to the normal saline group. The extract showed 60.9, 77.0 and 87.4% inhibition at the doses 50, 100 and 200 mg/kg, respectively.

DISCUSSION

The extract possesses a significant anti-inflammatory effect when compared to that of indomethacin 20 mg/kg. This result agreed with the findings of Duke (1992) that, flavonoids isolated from medicinal plants possess anti-nociceptive and/or anti-inflammatory effects. Other flavonoids potentially inhibit prostaglandins, a group of powerful pro-inflammatory signaling molecules (Manthey, 2000). Manthey *et al.* (2001) reported that flavonoids also inhibit phosphodiesterases involved in cell activation, the effects of which predominantly depend upon the biosynthesis of protein cytokines that mediate migration and diapedesis of circulating leucocytes to sites of injury. Protein kinases are another class of regulatory enzymes affected by flavonoids. Thus, the inhibition of these key enzymes provides the possible mechanism by which the extract of *Commiphora africana* acts on inflammation. The hydro-ethanolic stem bark extract of *Commiphora africana* significantly p<0.05 reduced the number of abdominal constrictions induced by acetic acid in mice. This activity resides more at higher dose of the extract (200 mg/kg body weight), which shows higher inhibition than that of piroxicam (20 mg/kg body weight),

revealing anti-nociceptive activities. The abdominal constriction response induced by acetic acid is very sensitive and able to detect anti-nociceptive effects of compounds/doses levels that may be inactive in tail-flick assay (Collier *et al.*, 1968; Bentely *et al.*, 1981). It is thought to involve in part, local peritoneal receptors (Bentely *et al.*, 1983), suggesting that the extract of *Commiphora africana* may interfere with such peritoneal receptors to bring about the observed analgesic effect. Since the extract of *Commiphora africana* is rich in flavonoids and also tannins the extract may be a good source of analgesic.

CONCLUSION

In conclusion the extract of *Commiphora africana* possesses significant anti-nociceptive and anti-inflammatory effects. This extract contains a strong analgesic and anti-inflammatory agents, which relates to its use locally as a remedy in wound healing.

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